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WO 2001/096322 A1 **WO 1998/031219 A1**
WO 1993/021992 A1 **US 5830526 A**

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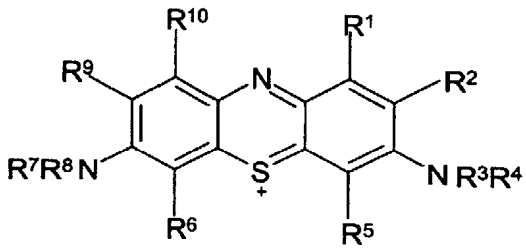
(54) Abstract Title
Phenothiazinium derivatives and their use to reduce pathogenic contaminants

(57) Phenothiazinium derivatives of formula I wherein R^1 to R^{10} are as defined in the application, methods for the preparation of these compounds, and the use of these compounds, in particular for reducing pathogenic contaminants, especially in blood.



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The derivatives may also be used or incorporated in pharmaceutical compositions, medical devices, woven and non-woven fibres, dyestuffs and surface coating materials. They may be used to produce light induced reactive oxygen species such as singlet oxygen.

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PHOTOSENSITIZERS AS ANTIMICROBIAL AGENTS

Field of the Invention

The present invention relates to compounds and methods for reducing the level of active pathogenic contaminants, such as viruses, bacteria and parasites. In particular, the present invention relates to photosensitizer compounds and methods for reducing the level of pathogens, particularly bacteria, found in whole blood and components of whole blood, particularly red blood cells.

Contamination of blood products with infectious microorganisms such as viruses and bacteria presents a serious health hazard for people handling or being transfused with blood, or various blood components such as platelets, red cells, blood plasma, blood proteins and other components isolated from blood. Although blood screening procedures exist, these procedures may miss contaminants because there are "window" periods of microorganism infection, normally straight after infection, in which the microorganism is undetectable.

Similarly, virucidal methods used to produce non-infectious plasma derivatives, such as heat, solvent-detergent treatment, and gamma irradiation are typically ineffective or too harsh to be used for decontaminating other blood components such as whole blood, red cells and platelets.

Decontamination treatments that inactivate contaminating pathogens but do not harm the cellular fractions of blood and are of low toxicity in the dosages employed are not

readily available. The use of photosensitizers, compounds which absorb light of a defined wavelength and transfer the energy to an acceptor molecule, i.e. an oxygen molecule to produce a reactive oxygen species, have been proposed for blood component sterilisation.

European patent application 0,196,515 relates to the use of non-endogenous photosensitizers such as porphyrin and phenothiazine derivatives for inactivating viruses.

A problem with known photosensitizers is that in the dosages employed they exhibit toxicity to mammalian systems and damage to cellular blood components may occur. For example, photochemicals such as the porphyrins have been shown to cause membrane damage in the presence of light and oxygen, which significantly reduces the survivability of the phototreated red cells during storage. Moreover, the fluidity of the cell membrane of red cells, which is essential for their passage through the channels in the spleen and liver, decreases rendering the cells unsuitable for transfusion.

Although, phenothiazinium-5-ium dyes such as methylene blue, toluidine blue O, thionine, azure A, azure B and azure C have been shown to inactivate animal viruses these particular dyes have certain disadvantages which limit their usefulness for inactivating pathogens in whole blood or blood components. For example, it has been shown that red cells take up or bind such dyes (Sass et al. J Lab. Clin. Med 73: 744-752 (1969)). Also, methylene blue treated red blood cells have shown to bind to plasma proteins, such as IgG and albumin (Wagner et al, Transfusion 33: 30-36 (1992)). Moreover, some of these

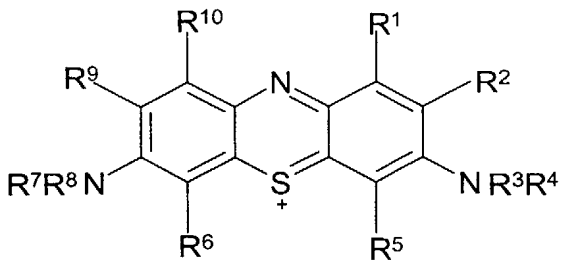
dyes do not readily cross the cell membrane of blood components and are so less effective at reducing the level of intracellular pathogens.

WO 98/31219 discloses the use of amphiphilic phenothiazinium dyes for inactivating pathogenic contaminants, such as viruses and bacteria frequently found in whole blood or blood components. This patent application does not disclose the preparation of any specific compounds. Moreover, the examples of this patent application focus on the use of 1,9-dimethyl methylene blue, a known phenothiazinium compound, as a virucidal agent.

The present invention therefore seeks to provide improved compounds, compositions and methods for reducing the level of active pathogenic contaminants, particularly bacterial contaminants, such as those found in whole blood and components of whole blood, particularly red blood cells.

Disclosure of the Invention

According to a first aspect of the invention, there is provided a compound of formula I,



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wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7 and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

provided that at least one of R^3, R^4, R^7 or R^8 represents hydrogen and that at least one of R^1, R^2, R^5, R^6, R^9 or R^{10} is other than hydrogen;

or a compound of formula I wherein

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7, and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$, or R^3 together with R^4 or R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, X is absent or represents $-CH_2-$, NR^{14}, O or S, and at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

provided that at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 form a ring system selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, S or O and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;or

$-N(R^4)-CH_2-CH=CH-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11}; or

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or

more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$; or

$-N(R^8)-CH_2-CH=CH-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

or a compound of formula I wherein

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7, and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

provided that at least one of R^3 together with R^4 and R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, X is absent or represents $-CH_2-$, NR^{14}, O or S, and at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

wherein R^{11}, R^{12} and R^{13} independently represent H or lower alkyl;

R^{14} independently represents H, lower alkyl or aryl, the latter group of which is optionally substituted with halo, lower alkyl and OR^{11};

but not including a compound of formula I wherein R^1, R^3, R^4, R^5, R^6, R^9 and R^{10} each represent hydrogen and R^2, R^7 and R^8 each represent methyl;

and not including a compound of formula I wherein R^1, R^2, R^5, R^6, R^7, R^8 and R^{10} each represent hydrogen and R^3, R^4 and R^9 each represent methyl;

and not including a compound of formula I wherein R^1, R^3, R^5, R^6, R^7 and R^{10} each represent hydrogen, R^2 and R^9 each represent methyl, and R^4 and R^8 each represent ethyl;

which compounds or a pharmaceutically acceptable derivative thereof are referred to together hereinafter as "the compounds of the invention".

Pharmaceutical acceptable derivatives include salts and solvates, such as acid addition salts. In other words, the compounds of the invention may include an anion, monovalent or polyvalent, sufficient to balance the charge on the amphiphilic phenothiazin-5-ium dye. Examples of such counter ions include non-organic moieties, such as halides i.e. chloride or bromide, sulfates and phosphates, and organic moieties, such as acetate, citrate and tartrate.

The term lower alkyl is intended to include linear or branched, cyclic or acyclic, C_1-C_{20} alkyl which may be interrupted by oxygen (preferably no more than five oxygen atoms are present in each alkyl chain). Lower alkyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} may represent and which R^{14} and the ring system formed from R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7, R^9 together with NR^7, R^3 together with R^4 and R^7 together with R^8 may be substituted include C_1-C_{18} alkyl, C_1-C_{17} alkyl, C_1-C_{16} alkyl, C_1-C_{15} alkyl, C_1-C_{14} alkyl,

C_1-C_{13} alkyl, C_1-C_{12} alkyl, C_1-C_{11} alkyl, C_1-C_{10} alkyl, C_1-C_9 alkyl, C_1-C_8 alkyl, C_1-C_7 alkyl, C_1-C_6 alkyl, C_2-C_6 alkyl and C_1-C_4 alkyl. Preferred lower alkyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} may represent and which R^{14} and the ring system formed from R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7, R^9 together with NR^7, R^3 together with R^4 and R^7 together with R^8 may be substituted include C_1, C_2, C_3, C_4, C_5 and C_6 alkyl. Particularly preferred lower alkyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10}, R^{11}, R^{12}, R^{13} and R^{14} may represent and which R^{14} and the ring system formed from R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7, R^9 together with NR^7, R^3 together with R^4 and R^7 together with R^8 may be substituted include C_1-C_3 alkyl, especially methyl or ethyl.

The terms lower alkenyl and lower alkynyl are intended to include linear or branched, cyclic or acyclic, C_2-C_{20} alkenyl and C_2-C_{20} alkynyl, respectively, each of which may be interrupted by oxygen (preferably no more than five oxygen atoms are present in each alkenyl or alkynyl chain). The term lower alkenyl also includes both the cis and trans geometric isomers.

Lower alkenyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} may represent include C_2-C_{18} alkenyl, C_2-C_{17} alkenyl, C_2-C_{16} alkenyl, C_2-C_{15} alkenyl, C_2-C_{14} alkenyl, C_2-C_{13} alkenyl, C_2-C_{12} alkenyl, C_2-C_{11} alkenyl, C_2-C_{10} alkenyl, C_2-C_9 alkenyl, C_2-C_8 alkenyl, C_2-C_7 alkenyl and C_2-C_6 alkenyl. Preferred lower alkenyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} may represent include C_2, C_3, C_4, C_5 and C_6 alkenyl.

Lower alkynyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} may represent include C_2-C_{18} alkynyl, C_2-C_{17} alkynyl, C_2-C_{16} alkynyl, C_2-C_{15} alkynyl, C_2-C_{14} alkynyl, C_2-C_{13} alkynyl, C_2-C_{12} alkynyl, C_2-C_{11} alkynyl, C_2-C_{10} alkynyl, C_2-C_9 alkynyl, C_2-C_8 alkynyl, C_2-C_7 alkynyl and C_2-C_6 alkynyl. Preferred lower alkynyl groups which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} may represent include C_2, C_3, C_4, C_5 and C_6 alkynyl.

Halo groups which R^1, R^2, R^5, R^6, R^9 and R^{10} may represent and with which R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9, R^{10} and R^{14} are optionally substituted or terminated, and with which the ring system formed from R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7, R^9 together with NR^7, R^3 together with R^4 and R^7 together with R^8 is optionally substituted, include fluoro, chloro, bromo and iodo.

By the term "aryl" which R^{14} may represent we include six to ten-membered carbocyclic aromatic groups, such as phenyl and napthyl which groups are optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11}.

For the avoidance of doubt, it will be appreciated that the compounds of the invention do not include methylene blue derivatives wherein R^3, R^4, R^7 and R^8 all represent methyl. The compounds of the invention also do not include Azure A, Azure B, Azure C, Thionin, Toluidine Blue O and new methylene blue. Moreover, it will be appreciated that the ring system as defined hereinbefore which R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7, R^9 together with NR^7, R^3 together with R^4 and R^7 together with

R^8 may form, is a non-aromatic ring system i.e. the ring system does not include a 4n + 2Π electron system.

By the terms "R^3 together with R^4 and/or R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m$" it will be appreciated that the exocyclic nitrogen atom forms part of the ring system. In other words, when R^3 together with R^4 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m$ wherein n and m both represent 2 and X is absent, then a pyrrolidine ring system is formed.

It will be appreciated that when R^2 together with NR^3, R^5 together with NR^3, R^6 together with NR^7 and/or R^9 together with NR^7 form a ring system as hereinbefore described, then we include 1,2,3,4-tetrahydropyridophenothiazinium compounds and 2,3-dihydropyrrolophenothiazinium compounds, and the tautomeric forms thereof.

Moreover, it will be appreciated that some of the compounds of the invention represent a narrow selection of the generic disclosure of WO 98/31219. Moreover, the compounds of the present invention suitably exhibit increased levels of activity for reducing the level of pathogenic contaminants, such as Gram-negative and/or Gram-positive bacteria, and/or the compounds of the present invention suitably exhibit decreased levels of toxicity to mammalian cells compared with the corresponding Azure A, B, C and TBO derivatives.

The compounds of the invention may exhibit tautomerism. All tautomeric forms and mixtures thereof are included within the scope of the invention.

The compounds to the invention may also contain one or more asymmetric carbon atoms and may therefore exhibit optical and/or diastereoisomerism. Diastereoisomers may be separated using conventional techniques e.g. chromatography or fractional crystallisation. The various stereoisomers may be isolated by separation of a racemic or other mixture of the compounds using conventional e.g. fractional crystallisation or HPLC, techniques. Alternatively, the desired optical isomers may be made by reaction of the appropriate optically active starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, for example with a homochiral acid followed by separation of the diastereometric esters by conventional means (e.g. HPLC, chromatography over silica). All stereoisomers are included within the scope of this invention.

Preferred compounds of the invention include those wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or C_1-C_4 alkyl, most preferably H or methyl.

Preferred compounds of the invention include those wherein R^3, R^4, R^7, and R^8 independently represent H or lower alkyl, preferably H or C_1-C_4 alkyl, especially H or methyl, or R^3 together with R^4 and/or R^7 together with R^8 form a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, and X is absent or represents CH_2, NR^{14}, O or S.

Preferably, R^{14} represents H or lower alkyl.

Preferred compounds of the invention include those wherein at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 form a ring system selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl or OR^{11}; or

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl or OR^{11}.

Preferably, the ring system formed by R^2 together with NR^3 or R^5 together with NR^3 is selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$, wherein at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more lower alkyl groups.

Preferably, the ring system formed by R^6 together with NR^7 or R^9 together with NR^7 is selected from the group having the formula:

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$, wherein at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more lower alkyl groups.

Alternative preferred compounds of the invention include those wherein at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 form a ring system selected from the group having the formula:

$-N(R^4)-CH_2-CH=CH-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more lower alkyl groups, preferably methyl groups; or

$-N(R^8)-CH_2-CH=CH-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more lower alkyl groups, preferably methyl groups.

It will be appreciated that the compounds of the invention may include one or more ring systems as hereinbefore described formed by the combination of R^2 together with NR^3; R^9 together with NR^7; R^2 together with NR^3 and R^9 together with NR^7; R^5 together with NR^3; R^6 together with NR^7; R^5 together with NR^3 and R^6 together with NR^7; R^2 together with NR^3 and R^6 together with NR^7; and, R^5 together with NR^3 and R^9 together with NR^7.

The compounds of the invention preferably include a single ring system as hereinbefore described formed by the combination of R^2 together with NR^3; R^5 together with NR^3; R^6 together with NR^7; or R^9 together with NR^7. A highly preferred single ring system is formed by the combination of R^2 together with NR^3 or R^5 together with NR^3, especially R^2 together with NR^3.

It will be appreciated that if a compound of the invention includes a single ring system as defined hereinbefore

formed by the combination of R^2 or R^5 together with NR^3 then R^7 together with R^8 may form a ring system of formula $-(CH_2)_n-X-(CH_2)_m$ as hereinbefore described. Similarly, if R^6 or R^9 together with NR^7 form a single ring system as defined hereinbefore, then R^3 together with R^4 may form a ring system of the formula $-(CH_2)_n-X-(CH_2)_m$ as hereinbefore described. Alternatively, in such compounds of the invention which include a single ring system formed by the combination of R^2 or R^5 together with NR^3 or R^6 or R^9 together with NR^7, then R^7, R^8 or R^3, R^4 respectively may independently represent H or lower alkyl.

Preferred compounds of the invention include those wherein:

R^1, R^6, R^9 and R^{10} independently represent H or methyl;

R^2, R^7 and R^8 each represent lower alkyl, preferably methyl;

R^5 together with NR^3 forms a ring system as defined hereinbefore; and

R^4 independently represents H or methyl, preferably H.

Alternative preferred compounds of the invention include those wherein:

R^1, R^5, R^6, R^9 and R^{10} independently represent H or methyl;

R^4 represents H or lower alkyl, preferably H;

R^7 represents H or methyl;

R^8 represents methyl; and

R^2 together with NR^3 forms a ring system as defined hereinbefore, preferably a ring system selected from $-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$, wherein a $-CH_2-$ group of each of said ring systems is optionally substituted with a lower alkyl group.

Preferred compounds of the invention include those wherein:

R^1, R^2, R^6, R^9 and R^{10} independently represent H or methyl;

R^4 represents H or lower alkyl, preferably H;

R^7 represents H or methyl;

R^8 represents methyl; and

R^5 together with NR^3 forms a ring system as defined hereinbefore, preferably a ring system selected from $-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$, wherein a $-CH_2-$ group of each of said ring systems is optionally substituted with a lower alkyl group.

Alternative preferred compounds of the invention include those wherein

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, lower alkyl, halo, or OR^{11};

R^3, R^4, R^7 and R^8 independently represent H or lower alkyl;

provided that at least one of R^3 together with R^4 and R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, and X is absent or represents NR^{14}, O or S.

Preferably, R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent H or lower alkyl, provided that at least one of R^3 together with R^4 and R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, and X is absent or represents NR^{14}, O or S, and provided that at least one of R^3, R^4, R^7 or R^8 represents H.

Further preferred compounds of the invention include those wherein:

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, lower alkyl or halo, preferably H or lower alkyl;
R^3 and R^4 both represent H; and
R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3,and X is absent or represents $-CH_2-$, NR^{14}, O or S.

Especially preferred ring systems which R^7 together with R^8 and/or R^3 together with R^4 may form include $-(CH_2)_2-X-(CH_2)_2-$ wherein X is as defined hereinbefore.

It will be appreciated that compounds of the invention also embrace compounds which do not include a ring system as hereinbefore defined. Such preferred compounds of the invention include those wherein:
R^3 represents H;
R^7 represents lower alkyl;
R^4 and R^8 independently represent H or lower alkyl; and
R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^2, R^5, R^6, R^9 or R^{10} is other than hydrogen.

Further preferred compounds of the invention include those wherein:
R^2, R^7 and R^8 each represent methyl;
R^3 and R^4 each represent H; and
R^1, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^5, R^6, R^9 and R^{10} is other than hydrogen.

Alternative further preferred compounds of the invention include those wherein:

R^3 and R^4 each represent H;

R^7 and R^8 each represent methyl; and

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^2, R^5, R^6, R^9 and R^{10} is other than hydrogen.

Alternative further preferred compounds of the invention include those wherein:

R^3 represents H;

R^4, R^7 and R^8 each represent methyl; and

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^2, R^5, R^6, R^9 and R^{10} is other than hydrogen.

Alternative further preferred compounds of the invention include those wherein:

R^3, R^4 and R^7 each represent H;

R^8 represents methyl; and

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^2, R^5, R^6, R^9 and R^{10} is other than hydrogen.

Alternative further preferred compound of the invention include those wherein:

R^2 and R^9 both represent methyl;

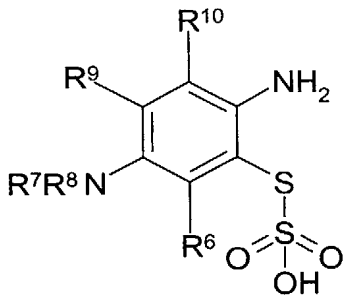
R^3 and R^7 both represent hydrogen;

R^4 and R^8 both represent ethyl; and

R^1, R^5, R^6 and R^{10} independently represent H, halo or lower alkyl, preferably H or methyl, provided that at least one of R^1, R^5, R^6 and R^{10} is other than hydrogen.

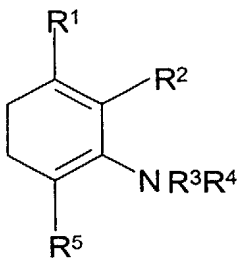
More preferred compounds of the invention include the compounds of Examples 1 to 10 described hereinafter, the structures of which are appended at the end of this application.

According to the invention there is also provided a process for the preparation of the compound of formula I as defined hereinbefore which comprises reacting a compound of formula II



II

wherein R^6, R^7, R^8, R^9 and R^{10} are as defined for a compound of formula I, with a compound of formula III



III

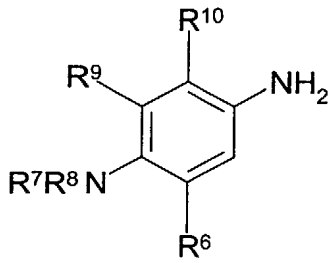
wherein R^1, R^2, R^3, R^4 and R^5 are as defined for a compound of formula I.

The reaction may be accomplished using methods which are well known to those skilled in the art. For example, the reaction may be accomplished by refluxing a mixture of the compound of formula II and III in an appropriate solvent, such as methanol, with silver carbonate on celite.

Alternatively, the compounds of the invention may be prepared by reaction of a compound of formula IV, as described hereinafter, with a compound of formula III. Typically, the reaction is performed in an aqueous acidic media with sodium sulfide and iron(III) chloride.

Alternatively, a 10*H*-phenothiazine may be oxidised to the phenothiazin-5-ium ion using an oxidant such as iodine in an inert solvent and the amine functionality of compounds of formula I added using primary or secondary amines at room temperature with stirring. Purification is achieved using planar or column chromatography.

A compound of formula II may be prepared from a compound of formula IV



IV

wherein R^6, R^7, R^8, R^9 and R^{10} are as previously defined for a compound of formula II. Typically the reaction is performed using the sulfate or other salt of the compound of formula IV in aqueous conditions in the presence of aluminium sulfate, sodium thiosulfate and zinc chloride followed by oxidation with, for example, potassium dichromate.

The compounds of formula IV and formula III and derivatives thereof, when neither commercially available nor subsequently described, may be obtained using conventional synthetic procedures in accordance with standard text books on organic chemistry or literature precedent, from readily accessible starting materials using appropriate reagents and reaction conditions.

For example, the compounds of formula IV when R^7 together with R^8 form a ring system of the formula $-(CH_2)_n-X-(CH_2)_m-$ as defined hereinbefore such as morpholine, pyrrolidine, piperidine, etc., may be synthesised by reaction of the appropriate ring system (i.e. morpholine) with a 4-halonitrobenzene, followed by subsequent reduction of the nitro group, for example with palladium on charcoal and ammonium formate in anhydrous methanol.

It will be appreciated by persons skilled in the art that, within certain of the processes described, the order of synthetic steps employed may be varied and will depend *inter alia* on factors such as the nature of other functional groups present in a particular substrate, the availability of key intermediates and the protecting group strategy (if any) to be adopted.

The protection and deprotection of functional groups may take place before or after any of the reaction steps described hereinbefore.

Protecting groups may be removed in accordance with techniques which are well known to those skilled in the art. The use of protecting groups is fully described in "Protective Groups in Organic Chemistry" edited by JWF McOmie, Plenum Press (1973) and "Protective Groups in Organic Synthesis", 2nd Edition, TW Greene PGM Wutz, Wiley- Interscience (1991).

Typically, the groups R^3, R^4, R^7 and R^8 of the compounds of formula I, II, III and IV as defined hereinbefore may need to be protected.

It will also be appreciated that various standard substituent or functional interconversions and transformations within certain compounds of formula I will provide other compounds of formula I.

The compounds of the invention represent derivatives of the known phenothiazin-5-ium dyes – Azure A, B and C and Toluidine Blue O (TBO).

Suitably, the compounds of the invention are photodynamic as they cause the formation of reactive oxygen species, such as singlet oxygen or oxygen free radicals, following irradiation with light of the appropriate wavelength in the presence of oxygen. Consequently, the compounds of the invention and compositions thereof as described hereinafter are suitable for use in both medical and non-

medical applications for which a photosensitizer/photodynamic agent is indicated.

Suitably, the compounds of the invention exhibit an increased level of activity, compared with the corresponding Azure A, B, C and TBO derivative, for reducing the level of active pathogens in a given composition. Such reduction may occur by rendering the pathogens inactive and/or non-infectious by reducing the number of pathogens in the composition.

By the term "pathogen" we include: viruses, such as retroviruses (e.g. HIV-1 and 2, HTLV), hepatitis viruses, adenoviruses, papovaviruses, herpes viruses and paramyxoviruses; bacteria, such as Gram-negative and Gram-positive organisms, for example Gram-negative bacilli (e.g. Escherichia coli, Pseudomonas aeruginosa, Yersinia enterocolitica), Gram-positive bacilli (e.g. Corynebacterium and Clostridium species), acid fast bacilli (e.g. a typical Mycobacterium), Gram-positive cocci (e.g. Streptococci and Staphylococci such as Staphylococcus epidermidis and methicillin-resistant Staphylococcus aureus), Gram-negative cocci (e.g. Neisseria), and protozoal infections, such as malaria, amoebiasis, trypanosomiasis, babesiosis and toxoplasmosis.

In particular, the compounds of the present invention suitably exhibit increased levels of activity for reducing the level of Gram-negative and/or Gram-positive bacteria in a given composition, compared with the corresponding Azure A, B, C and TBO derivative.

Suitably, the compounds of the invention exhibit decreased levels of toxicity towards mammalian cells, compared with the corresponding Azure A, B, C and TBO derivatives, Methylene Blue and Dimethyl Methylene Blue.

Suitably, the increased level of antipathogenic activity and/or decreased levels of toxicity of the compounds of the present invention opens the way for the reduction of pathogenic contaminants in both medical and non-medical applications, such as reducing pathogenic contaminants of whole blood or components of whole blood and the prophylactic and/or curative treatment of medical conditions susceptible to pathogenic infections, and the prophylactic disinfection of carriers, such as hospital workers, who may be exposed to such pathogens.

Moreover, the compounds of the invention and compositions described hereinafter are suitable for use in the curative and/or prophylactic treatment of a medical condition for which a photosensitizer/photodynamic agent is indicated. Preferably, the compositions of the invention are suitable for reducing pathogenic infections, such as in wounds/skin lesions caused by injury or surgery, infections causing abscesses, cysts, arthritis, urinary tract infections, pancreatitis, pelvic inflammatory disease, peritonitis, prostatitis, ulcers, the curative and/or prophylactic treatment of atherosclerosis; cataracts; restenosis; endometrial ablation; cancers such as bladder cancer; other proliferative diseases; fungal infections such as *Candida albicans*, *Aspergillus* and *Blastomyces*; viral, bacterial and protozoal infections as mentioned hereinbefore. The cytotoxicity of the photodynamic therapy may kill the infecting cells (in the case of bacteria or

fungi) or the affected host cells (in the case of viral infections).

In a further aspect, the present invention provides a composition comprising an insoluble support and a compound of the invention of formula I. Preferably, the insoluble support is also biocompatible.

The amount of the compound of the invention in the composition of the invention may vary over a wide range, depending on the activity of the compound of the invention and on the desired level of photodynamic/antipathogenic activity. Suitably the compound of the invention is present in an amount of greater than or equal to 0.01%, preferably greater than or equal to 0.1%, more preferably 0.5% by weight of the insoluble support. Suitably the composition of the invention is present in an amount of less than or equal to 10%, preferably less than or equal to 5%, more preferably less than or equal to 3% by weight of the insoluble support. Proportions in the range of 0.1% to 1% by weight of the compound of the invention to the weight of the insoluble support are especially preferred.

By the term "insoluble" we include that the support does not dissolve in aqueous solution at room temperature under atmospheric pressure over the intended timescale for photodynamic ability (i.e. the reactive oxygen species production) of the photosensitizing compound. By the term "biocompatible" we include that the support is in a form that does not produce an adverse, allergic or other untoward reaction to a mammalian system when it is used in accordance with the present invention.

Preferably, the insoluble support comprises a polymer such as polyurethane resins; polyethylene resins; polypropylene resins; polystyrene resins; polyacrylamide resins; polyacrylate resins; polymethacrylate resins; polyvinyl chloride resins; polyethylene terephthalate resins and/or polyamide resins.

The insoluble support may be flexible or a rigid support. Preferably, the insoluble support is coated and/or impregnated with the compound of the invention, either by physical mixture or copolymerisation. Alternatively, or additionally, the compound of the invention may be fixed to the surface of the insoluble support, optionally via a covalent bond.

For example, a polymer may be dyed using a solution of the compound of the invention in a suitable solvent. The polymer may for example be in the form of a fibre or film. The polymer may be a natural polymer, for example cotton, or a synthetic polymer as described hereinbefore. Alternatively, the compounds of the invention may be included in a polymeric melt or dope which is then formed by casting, moulding or extrusion to produce the desired article. The compounds of the invention may be dissolved or dispersed in the melt or dope. Such methods of casting, moulding and extrusion are well known in the field of polymer manufacture. The compounds of the invention may be bonded to a polymer to form a photosensitive polymer. Such polymers may be made by the inclusion of one or more such photosensitive monomers for example an addition polymerisation, so as to incorporate the photosensitiser in the polymer. Such polymers may alternatively be made by reacting a compound of the invention with a polymer,

thereby grafting the compound of the invention onto a polymer, for example in the form of dependent groups.

The flexible insoluble support may be formed into films, such as polymer films, with singlet oxygen producing ability for use in antipathogenic coatings. The films are suitable for packaging containers for foods, packaging materials for medical use, for example, materials for medical equipment and accessories such as bags for storing whole blood or components of whole blood. Alternatively, the insoluble supports may be rigid and used to form surfaces, such as domestic worktops, surfaces in hospitals, such as walls, floors, ceilings, woodwork or other clinical surfaces such as operating tables.

Alternatively, the composition of the present invention comprising an insoluble support and a compound of the present invention may be formed into fibre or pellets. The fibres may be converted into woven, knitted or non-woven textile articles such as cleaning cloths, wipes, surgical gowns, bed linen, wound dressings and bandages. The wound dressing may be exposed to light before and/or during and/or after application to a wound thereby producing a dressing which reduces and/or prevents and/or eliminates pathogenic contaminants.

It will be appreciated that a feature of the composition of the present invention is that they may provide a surface which may reduce and/or prevent and/or eliminate pathogenic contaminants, such as bacteria, upon exposure of the surface to light of the appropriate wavelength and intensity.

Alternatively, the compositions of the invention may be fabricated into or coated on various medical devices and surgical implants, such as venous, urinary or balloon catheters, breathing tubes, vascular stents, intraocular lenses, orthopaedic implants, other artificial implants, interfaces, artificial joints, surgical screws and pins. Preferably, the coating partially or totally coats the surface of the medical device or is an intrinsic ingredient of the polymeric component of the device.

The medical devices enable the compounds of the invention to be delivered to a particular target tissue. For example, in the treatment of atheroscheloris/restenosis the target cells are the smooth muscle cells on the blood vessel lumen surface, and inflammatory cells such as macrophages. This site specific delivery reduces or substantially eliminates considerable side-effects associated with photodynamic agents residing in and accumulating in non-target tissues. This increases patient compliance as a patient who has been treated with a medical device of the present invention only needs to shield the treated tissue from exposure to light, rather than other parts of the body which may be necessary with photodynamic agents having no site specific delivery action. It will be appreciated that the medical devices may be delivered to the target tissue by methods well known to those skilled in the art.

The insoluble supports can be produced by methods well known to those skilled in the art such as casting methods, extrusion methods and drawing methods.

It will also be appreciated that the compounds of the present invention may be formulated by techniques well known to those skilled in the art into dyestuffs, such as paints and inks, and surface coatings, for coating for example glass, ceramics, plastics, metals and building materials, such as bricks, concrete and woodwork. Advantageously, such formulations allow the compounds of the present invention to be applied to internal and/or external surfaces of various articles where it is desirable to have a hygienic coating i.e. a coating which reduces and/or prevents and/or eliminates pathogenic contaminants. Examples of such articles include the walls, floors and ceilings of hospital, abattoirs and clean rooms in scientific laboratories, as well as external building surfaces such as tunnels. The formulations may be applied to such articles by techniques well known to those skilled in the art such as by painting and spraying the desired surface with the formulation.

In yet a further aspect, the present invention provides a process for preparing the compositions of the invention comprising a photosensitizing compound and the insoluble support.

Typically, in both medical and non-medical applications absorption of light of the appropriate wavelength, preferably 600 to 700 nm and appropriate intensity in the range normally less than or equal to 100 J/cm^2, the compounds and compositions of the present invention cause the formation of reactive oxygen species, such as singlet oxygen, which typically travel a distance of less than 0.1 μm depending on the composition of the medium surrounding the compound/composition of the present invention. It will

be appreciated that light of the appropriate wavelength may be provided by, for example, sunlight or another light source such as a helium-neon or tuneable laser or a Paterson Lamp (Phototherapeutics Ltd., Manchester, UK) or a flat light box containing fluorescent strip lights to up-illuminate blood bags.

Consequently, in non-medical applications where, for example, the compositions of the present invention are fabricated into work surfaces or applied to the external and/or internal surface of an article, the treated surfaces suitably cause the formation of reactive oxygen species on irradiation with sunlight and therefore exhibit antimicrobial activity. It is an advantage of the present invention that no additional equipment is required so that such compositions induce antimicrobial activity.

According to a further aspect, the compounds of the invention are suitable for use in medicine. In particular, the compounds of the invention are suitable for use in the curative and/or prophylactic treatment of a medical condition for which a photosensitizer/photodynamic agent is indicated.

In particular, the compounds of the invention suitably are useful for the prophylactic and/or curative treatment of a disease state susceptible to pathogenic infection, such as wounds, cuts and skin lesions resulting from injury or surgery, and internal infection where the colonisation site is amenable to light delivery, such as the eradication of Helicobacter pylori infection of the gastric mucosa.

Thus according to a further aspect of the invention, there is provided a pharmaceutical composition comprising a compound of the invention in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier, e.g. Cremophor EL.

Preferably, the compounds of the invention will be administered topically in the form of the pharmaceutical composition in a pharmaceutically acceptable dosage form. Suitable pharmaceutical acceptable dosage forms are about 0.1-0.5 weight % of the compound of the invention. However, it will be appreciated that other methods of administration such as subcutaneous, intravenous, intra-arterial, transdermal, intranasal and inhalation are embraced by the present invention.

It will be appreciated that once the pharmaceutical preparation has been applied to the target tissue it can be irradiated with an external light source such as a helium-neon or tuneable laser, Paterson lamp or white light source and/or by sunlight so that the reactive oxygen species are generated.

According to a further aspect, the present invention provides a method for reducing the level of a pathogenic contaminant in whole blood and/or a component of whole blood comprising delivering a compound or composition of the present invention to a whole blood and/or a component of whole blood and irradiating the compound or composition with light sufficient to generate singlet oxygen.

By the term "component of whole blood" we include one or more components that may be separated from whole blood for

example: cellular blood components, such as red blood cells and platelets; blood proteins, such as blood clotting factors, enzymes, albumin, plasminogen and immunoglobulins; and, liquid blood components, such as plasma and plasma-containing compositions. Compositions containing a cellular blood compound and/or a blood protein may optionally be leukodepleted i.e. the concentration of leukocytes in the composition has been reduced by a specific amount, such as a factor of 10^5.

Suitably, the compounds and composition of the present invention inactivate contaminating pathogens present in whole blood and/or a component of whole blood upon irradiation with light sufficient to generate singlet oxygen. Moreover, the compounds of the invention typically exhibit increased antipathogenic activity and lower toxicity to whole blood and/or a component of whole blood compared with the corresponding known TBO, Azure A, B or C derivative.

Thus the compounds of the invention can typically be employed in lower dosages (e.g.1-5 μM) compared with the known compounds. This coupled with the fact that the compounds of the invention typically show reduced membrane damage to cellular blood components compared with the known compounds, means that the resultant treated cells are suitable for transfusion to a patient.

It will be appreciated that whole blood may be decontaminated (i.e. a reduction of pathogenic activity) according to the method of the present invention. Alternatively, the whole blood may first be separated into blood components, such as plasma, platelets and red blood

cells by methods well known to those skilled in the art and then these blood components decontaminated according to the method of the present invention.

Preferably, the compounds of the invention are used for reducing the level of Gram-positive and Gram-negative bacteria, such as Staphylococcus epidermidis and Yersinia enterocolitica, in whole blood and/or a blood component of whole blood. More preferably, the compounds of the invention are used for reducing the level of Gram-positive and Gram-negative bacteria in platelets and red blood cells respectively. Suitably, the compounds of the invention exhibit increased activity at reducing levels of Gram-positive and Gram-negative bacteria compared with the known corresponding TBO, Azure A, B or C derivatives. Furthermore, the compounds of the present invention typically show lower levels of cell membrane damage of blood components compared with the known corresponding TBO, Azure A, B or C derivatives. Still further, the compounds of the present invention suitably absorb light of wavelengths not typically absorbed by the haem pigments of red blood cells i.e. < 620 nm.

In a typical procedure, blood is centrifuged for a sufficient time and a sufficient centrifugal force to sediment the red blood cells. Leukocytes collect primarily at the interface of the red cells and the plasma-containing supernatant in the buffy coat region. The supernatant, which contains plasma, platelets, and other blood components, may then be removed and centrifuged at a higher centrifugal force, whereby the platelets sediment.

Human blood normally contains about 7×10^9 leukocytes per litre. The concentration of leukocytes, which pellet with the red cells, can be decreased if desired by filtering through a filter that decreases their concentration by selected orders of magnitude. Leukocytes can also be removed from each of the components by filtration through an appropriate filter that removes them from the solution.

In a preferred embodiment of this invention, the whole blood or blood component to be decontaminated is obtained in, prepared in or introduced into a gas permeable blood preservation bag that includes a compound and/or composition of the present invention (i.e. the compound in powder form). The bag is scaled and flattened to a width sufficiently narrow to permit light to irradiate the contents, such that any pathogenic contaminant present in the blood or blood component in the bag will be irradiated. Any such blood bag known to those skilled in the art may be used provided that the bag is transparent to the selected wavelength of light.

Alternatively, or additionally the blood or blood component to be decontaminated may be introduced into a gas permeable bag fabricated from an insoluble polymeric composition of the present invention. In other words, the blood bag itself includes a compound of the present invention which may therefore mean that none of or a reduced level of a compound of the invention needs to be added to the whole blood and/or blood component. This method suitably further reduces the toxicity of the treated blood component upon transfusion to a mammal. Preferably the gas permeable blood preservation bag also contains oxygen.

Preferably, an effective concentration of the compound of the invention added to the whole blood or component of whole blood is at least 0.2 μM, more preferably at least 1μM. Preferably, an effective concentration of the compound of the invention used is not greater than 50 μM, more preferably not greater than 25 μM. A most preferred concentration of the compound of the invention used in the method is 10 μM.

The composition that is to be decontaminated is irradiated with light of the appropriate wavelength (620-650 nm) for an appropriate time (ca. 5 minutes) giving an appropriate light dose (ca. 15 J/cm^2), most preferably, employing a flat white light box. Suitably, the compounds of the present inventions generate reactive oxygen species which reduce the level of pathogenic contaminants in the composition, thereby rendering the composition suitable for transfusion to a patient.

In yet a further aspect, the present invention provides a method of producing light-induced singlet oxygen which comprises irradiating a compound or composition of the invention with light of the appropriate wavelength, preferably 620-650 nm, in the presence of oxygen.

Preferred embodiments of the invention will now be described by way of the following non-limiting examples.

Example 1

2-Amino-5-dimethylaminophenylthiosulfonic acid

N,N-Dimethyl-*p*-phenylenediamine sulfate (30.3g, 130 mmol) was added to a mechanically stirred solution of aluminium sulfate octadecahydrate/water (43.6g, 65mmol/100ml). To this was added sodium thiosulfate/water (22.0g, 139mmol/80ml) followed by zinc chloride/water (8.8g, 63mmol/12ml). The solution was cooled to 0°C and potassium dichromate/water (5.0g, 17mmol/20ml) was added dropwise over a 30 minute period. Following this addition, the mixture was allowed to stir for 2 hours. During the last 29 minutes the temperature was allowed to rise to 10°C causing the formation of a viscous precipitate. This was isolated by filtration and washed with water followed by acetone. 2-Amino-5-dimethylaminophenylthiosulfonic acid, yield=15.87g (49%), m.p. 190°C (dec.)

Example 2

3-Amino-7-dimethylamino-1-methylphenothiazinium sulfate

2-Amino-5-dimethylaminophenylthiosulfonic acid of Example 1 (1.0g, 4mmol) and *m*-toluidine (0.54g, 5mmol) were refluxed in 120ml methanol and silver carbonate on celite (5g, 50% w/w) added slowly over 0.5h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue was extracted with dicholoromethane and purified by medium pressure liquid chromatography. The product was obtained as a dark purple microcrystalline powder (21%), m.p. 187°C. Found C 50.45; H 4.55; N 11.89; S 16.88%. $C_{15},H_{17}N_3S_2O_4$ requires C 49.03; H 4.66; N 11.43; S 17.45%.

Example 3

3-Methyl-2-amino-5-dimethylaminophenylthiosulfonic acid

2-Methyl-*N,N*-dimethyl-*p*-phenylenediamine sulfate (32.2g, 130mmol) was added to a mechanically stirred solution of aluminium sulfate octadecahydrate/water (43.6g, 65mmol/100ml). To this was added sodium thiosulfate/water (22.0g, 139mmol/80ml) followed by zinc chloride/water (8.8g, 63mmol/12ml). The solution was cooled to 0°C and potassium dichromate/water (5.0g, 17mmol/20ml) was added dropwise over a 30 minute period. Following this addition, the mixture was allowed to stir for 2 hours. During the last 20 minutes the temperature was allowed to rise to 10°C causing the formation of a viscous precipitate. This was isolated by filtration and washed with ice-water followed by ice-cool acetone. 3-Methyl-2-amino-5-dimethylaminophenylthiosulfonic acid, yield=14.06g (41%), m.p. 176°C (dec.)

Example 4

3-Amino-7-dimethylamino-1,9-dimethylphenothiazinium sulfate

3-Methyl-2-amino-5-dimethylaminophenylthiosulfonic acid of Example 3 (1.05, 4mmol) and *m*-toluidine (0.54g, 5mmol) were refluxed in 120ml methanol and silver carbonate on celite (5g, 50% w/w) added slowly over 0.5h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue wax extracted with dichloromethane and purified by medium pressure liquid chromography. The product was obtained as a purple microcrystalline powder (18%), m.p. 191°C. Found C 51.05; H 5.15; N 11.30; S 16.38%. $C_{16}H_{19}N_3S_2O_4$ requires C 50.38; H 5.02; N 11.02; S 16.81%.

Example 5

9-Dimethylamino-1,2,3,4-tetrahydropyrido[3,2-*b*]phenothiazinium sulfate

2-amino-5-dimehtylaminophenylthiosulfonic acid of Example 1 (1.0g, 4mmol) and 1,2,3,4-tetrahydroquinoline (0.67g, 5mmol) were refluxed in 130ml methanol and silver carbonate on celite (5g, 50% w/w) added slowly over 0.5h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue was extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a dark purple microcrystalline powder (28%) m.p. 167°C (dec.). found C 51.66; H 4.71; N 10.55; S 16.05%. $C_{17}H_{19}N_3S_2O_4$ requires C 51.89; H 4.87; N 10.68; S 16.30%.

Example 6

8-Dimethylamino-2,6-dimethyl-2,3-dihydropyrrolo[3,2-*b*]phenothiazinium sulfate

2-amino-5-dimethylaminophenylthiosulfonic acid of Example 1 (1.0 g, 4 mmol) and 2-methylindoline (0.67 g, 5 mmol) were refluxed in 150 ml methanol and silver carbonate on celite (5 g, 50% w/w) added slowly over 0.5 h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue was extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a black amorphous powder (19%) m.p. 220°C (dec.). found C 51.95; H 4.90; N 10.35; S 15.65%. $C_{18}H_{21}N_3S_2O_4$ requires C 53.05; H 5.19; N 10.31; S 15.73%.

Example 7

3-Amino-7-dimethylamino-1,2-dimethylphenothiazinium sulfate

2-Amino-5-dimethylaminophenylthiosulfonic acid of Example 1 (1.0 g, 4 mmol) and 1,2-dimethylaniline (0.61 g, 5 mmol) were refluxed in 120 ml methanol and silver carbonate on celite (5 g, 50% w/w) added slowly over 0.5 h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue wax extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a purple/black powder (38%), m.p. 180°C. Found C 51.10; H 4.74; N 10.80; S 16.88%. $C_{16}H_{19}N_3S_2O_4$ requires C 50.38; H 5.02; N 11.02; S 16.81%.

Example 8

3-Amino-7-dimethylamino-2,4,9-trimethylphenothiazinium sulfate

3-Methyl-2-amino-5-dimethylaminophenylthiosulfonic acid of Example 3 (1.05 g, 4 mmol) and 2,6-dimethylaniline (0.61 g, 5 mmol) were refluxed in 120 ml methanol and silver carbonate on celite (5 g, 50% w/w) added slowly over 0.5 h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue wax extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a blue-black amorphous powder (12%), m.p.201-2°C. Found C 50.33; H 5.20; N 11.00; S 15.90%. $C_{17}H_{21}N_3S_2O_4$ requires C 51.62; H 5.35; N 10.62; S 16.21%.

Example 9

3-Amino-7-dimethylamino-2,9-dimethylphenothiazinium sulfate

3-Methyl-2-amino-5-dimethylaminophenylthiosulfonic acid of Example 3 (1.05 g, 4mmol) and o-toluidine (0.54 g, 5 mmol) were refluxed in 120 ml methanol and silver carbonate on celite (5 g, 50% w/w) added slowly over 0.5 h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue wax extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a purple crystals (23%), m.p. 196-7°C. Found C 49.15; H 4.76; N 10.67; S 16.95%. $C_{16}H_{19}N_3S_2O_4$ requires C 50.38; H 5.02; N 11.02; S 16.81%.

Example 10

3-Amino-7-dimethylamino-1,4-dimethylphenothiazinium sulfate

2-Amino-5-dimethylaminophenylthiosulfonic acid of Example 1 (1.05 g, 4 mmol) and 2,5-dimethylaniline (0.61 g, 5 mmol) were refluxed in 120 ml methanol and silver carbonate on celite (5 g, 50% w/w) added slowly over 0.5 h. The reaction mixture was refluxed for a further hour, filtered through a celite pad and the filtrates evaporated. The residue wax extracted with dichloromethane and purified by medium pressure liquid chromatography. The product was obtained as a black amorphous powder (11%), m.p. 200-2°C. Found C 50.85; H 5.15; N 10.70; S 15.38%. $C_{16}H_{19}N_3S_2O_4$ requires C 50.38; H 5.02; N 11.02; S 16.81%.

Example 11 - Antimicrobial activity in whole blood

The compounds of the invention were used as a photosensitiser in 4.5 cm petri dishes at a range of concentrations, 32-0.25 μM, in 10 ml of whole blood and an inoculum of Yersinia enterocolitica at 10^2 organisms ml^{-1}, using the Paterson lamp as the light source. For each petri dish illumination was carried out for 167 s (light dose = 6.3 J/cm^2), the dish grown on and subsequently plated out on horse blood agar and colonies enumerated. For each photosensitizer repeat experiments were carried out to give $n \geq 6$.

Results for the above showed that the compounds of the invention were photobactericidal.

Example 12 - Photomicrobial Activity

The photobactericidal efficacies of a range of compounds of the invention as disclosed in the preceding Examples were measured against a Gram positive and a Gram negative organism, namely Staphylococcus epidermidis and Escherichia coli. Comparative experiments were performed using the known Azure A and TBO compounds.

Both bacterial strains were grown and then diluted to a concentration of 10^6 colony-forming units/ml. Aliquots of the strains were then incubated for 18 hours at 37 °C in microtitre trays with various concentrations of the photosensitizers giving a final range of 80-2.5 μM, with zero photosensitizer concentrations in each case for control purposes. The trays were then either illuminated for 0.5 hour using an array of white strip lights giving a

light dose of 6.3 J/cm^2 or foil-covered (dark controls). From each well showing inhibition of growth 1µl was sub-cultured on horse blood agar and incubated for 18 hours at 37 °C. Minimum lethal concentrations were then determined as the lowest concentration for each photosensitizer giving no bacterial growth. Results are given in Table 1 below.

Table 1

	Min. Lethal Conc. (µM)			
	S. Epidermidis		E. coli	
	Light	Dark	Light	Dark
TBO	20	40	40	80
AA	40	>80	>80	>80
Example 7	10	20	20	80
Example 8	<2.5	5	2.5	10
Example 4	<2.5	10	5	40
Example 2	5	10	10	40
Example 9	10	40	10	40
Example 6	<2.5	5	2.5	20

The results demonstrate that the compounds of the invention when irradiated with light exhibit substantially greater antibactericidal activity than azure A and TBO against both strains of bacteria. Moreover, the compounds of the invention exhibit increased antibactericidal activity compared with the corresponding known TBO and Azure A counterparts in the absence of light.

Example 13 - Toxicities

In order to investigate the mammalian toxicities, the mouse mammary tumour cell line EMT-6 was employed. 96 well microtitre plates were seeded with 1000 cells per well (in 200 μl RPMI 1640 culture medium (Life Technologies, Paisley, UK)) and incubated at 37°C, 5% CO_2 : 95% air for 2 days. Varying concentrations of the compounds of the invention as disclosed in the preceding Examples (0-160 μm) were added and the cells incubated at 37 dcμm) were added and the cells incubated at 37°C in 5% CO_2 : 95% air for 3 hours. The medium containing the compounds of the invention as disclosed in the preceding Examples was then aspirated and the cells rinsed with 200 μl RPMI 1640, before replacing with a further 200 μl RPMI 1640. The cells were then grown again at 37°C, 5% CO_2 : 95% air for a further 3 days. To evaluate cell viability and thus calculate percentage toxicity, 25 μl MTT - 3-[4,5-dimethylthiazol-2yl]-2,5-diphenyl-2H-tetrazolium bromide - (5 mg ml^{-1}) was added to each well and this was incubated at 37°C, 5% CO_2 : 95% air, for 5 hours. The medium and MTT were aspirated, taking care not to disturb the formazan crystals, leaving approximately 30 μl in each well. 200 μl DMSO were then added to each well to solubilise the crystals. The plates were shaken for 10 minutes and the absorbance read on a plate reader (Anthos HT111, measuring filter 540 nm; reference filter 620 nm).

Comparative toxicity studies were performed for Azure A, toluidine blue and dimethyl methylene blue.

The results are given in Table 2.

Table 2

	% Toxicity at 40 μM
Azure A	47
Toluidine Blue	100
Dimethyl Methylene Blue	56
Example 4	2
Example 2	35

The toxicity is given at 40 μm as LD50 values were not reached for the compounds of the invention.

The results demonstrate that the compounds of the invention (i.e. Example 2 and Example 4 described hereinbefore) are substantially less toxic to mammalian cells than the known Azure A, toluidine blue and dimethyl methylene blue compounds.

Example 14

Dyeing of a cellulose film with the compound of Example 2

The compound of Example 2 (1 mg) was dissolved in a solution comprising 6 parts methanol and 5 parts water (10 ml) and a cellulose film (thickness of 50 microns, dimensions of 2 cm^2) immersed in the solution. After immersion for 12 hours at room temperature or 10 minutes at reflux, the film was washed with methanol and dried between filter papers. Spectrophotometric analysis showed that the cellulose film had been impregnated with the compound of Example 2.

Example 15

Preparation of an insoluble support comprising the compound of Example 2

A 50% w/w dispersion of polyurethane (230 ml) and the compound of Example 2 (90 mg) in 20 ml water were stirred vigorously for 15 minutes using an overhead stirrer. The pale blue solution was then poured onto clean glass plate and allowed to dry over a period of 5 days at room temperature. The resulting film was photobactericidal against both Gram-positive and Gram-negative bacteria.

The reader's attention is directed to all papers and documents which are filed concurrently with or previous to this specification in connection with this application and which are open to public inspection with this specification, and the contents of all such papers and documents are incorporated herein by reference.

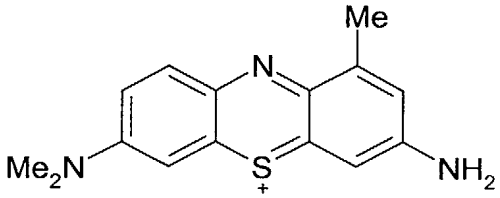
All of the features disclosed in this specification (including any accompanying claims, abstract and drawings), and/or all of the steps of any method or process so disclosed, may be combined in any combination, except combinations where at least some of such features and/or steps are mutually exclusive.

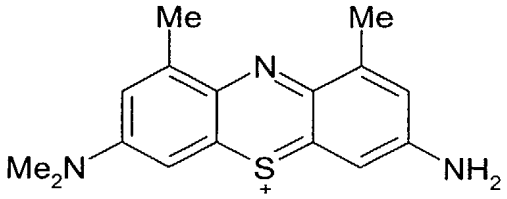
Each feature disclosed in this specification (including any accompanying claims, abstract and drawings), may be replaced by alternative features serving the same, equivalent or similar purpose, unless expressly stated otherwise. Thus, unless expressly stated otherwise,

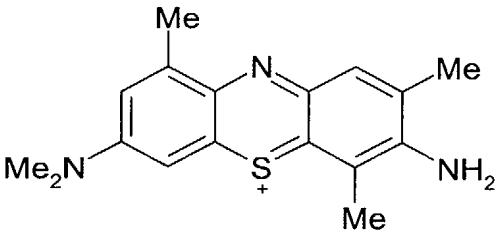
each feature disclosed is one example only of a generic series of equivalent or similar features.

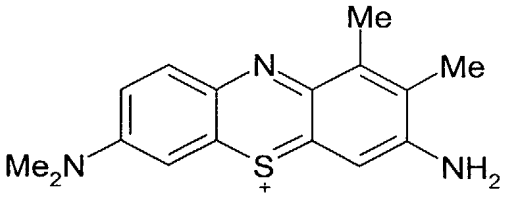
The invention is not restricted to the details of the foregoing embodiment(s). The invention extend to any novel one, or any novel combination, of the features disclosed in this specification (including any accompanying claims, abstract and drawings), or to any novel one, or any novel combination, of the steps of any method or process so disclosed.

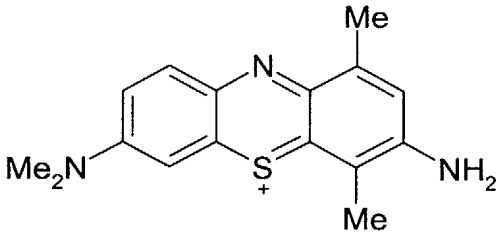
Specific Compounds of the Invention as disclosed in Examples 1 to 10

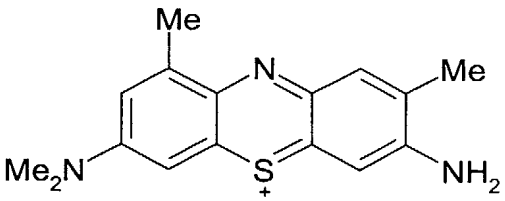


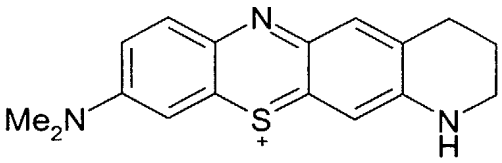


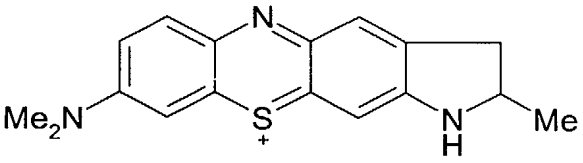












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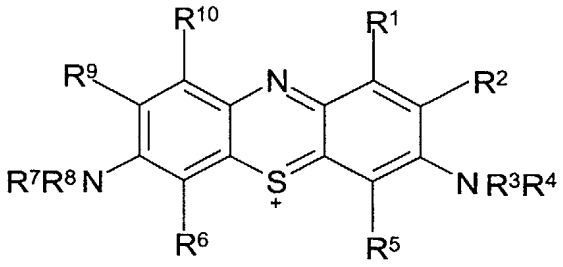
Claims

1. A compound of formula I



I

wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7 and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

provided that at least one of R^3, R^4, R^7 or R^8 represents hydrogen and that at least one of R^1, R^2, R^5, R^6, R^9 or R^{10} is other than hydrogen;

or a compound of formula I wherein

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7, and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which

are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$, or R^3 together with R^4 or R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, X is absent or represents $-CH_2-$, NR^{14}, O or S, and at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

provided that at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 form a ring system selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, S or O and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;or

$-N(R^4)-CH_2-CH=CH-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11}; or

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$; or

$-N(R^8)-CH_2-CH=CH-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

or a compound of formula I wherein

R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, OR^{11}, halo, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

R^3, R^4, R^7, and R^8 independently represent H, lower alkyl, lower alkenyl or lower alkynyl, the latter three of which are optionally substituted or terminated by one or more substituents selected from halo, lower alkyl, OR^{11} and $NR^{12}R^{13}$;

provided that at least one of R^3 together with R^4 and R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3, X is absent or represents $-CH_2-$, NR^{14}, O or S, and at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl and OR^{11};

wherein R^{11}, R^{12} and R^{13} independently represent H or lower alkyl;

R^{14} independently represents H, lower alkyl or aryl, the latter group of which is optionally substituted with halo, lower alkyl and OR^{11};

but not including a compound of formula I wherein R^1, R^3, R^4, R^5, R^6, R^9 and R^{10} each represent hydrogen and R^2, R^7 and R^8 each represent methyl;
and not including a compound of formula I wherein R^1, R^2, R^5, R^6, R^7, R^8 and R^{10} each represent hydrogen and R^3, R^4 and R^9 each represent methyl;
and not including a compound of formula I wherein R^1, R^3, R^5, R^6, R^7 and R^{10} each represent hydrogen, R^2 and R^9 each represent methyl, and R^4 and R^8 each represent ethyl.

2. A compound as claimed in claim 1 wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H, halo, or lower alkyl, the latter group is optionally substituted or terminated by one or more substituents selected from halo, OR^{11} and $NR^{12}R^{13}$.

3. A compound as claimed in claim 1 or 2 wherein R^3, R^4, R^7 and R^8 independently represent H or lower alkyl, or R^3 together with R^4 and/or R^7 together with R^8 form a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ wherein n and m independently represent 1, 2 or 3 and X is absent or represents NR^{14}, O or S.

4. A compound as claimed in claim 2 or 3 wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent H or C_1-C_4 alkyl.

5. A compound as claimed in claim 4 wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} independently represent H or methyl.

6. A compound as claimed in any one of the preceding claims wherein R^{14} represents H or lower alkyl.

7. A compound as claimed in any one of the preceding claims wherein at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 form a ring system selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl or OR^{11}; or

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$ in the case R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ groups of the ring system is optionally replaced by NR^{14}, oxygen or sulfur and/or at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more substituents selected from halo, lower alkyl or OR^{11}.

8. A compound as claimed in claim 7 wherein at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 forms a ring system selected from the group having the formula:

$-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more lower alkyl substituents; or

$-N(R^8)-(CH_2)_2-$ or $-N(R^8)-(CH_2)_3-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ groups of the ring system is optionally substituted by one or more lower alkyl substituents.

9. A compound as claimed in any one of claims 1 to 6 wherein at least one of R^2 together with NR^3 or R^5 together with NR^3 and/or at least one of R^6 together with NR^7 or R^9 together with NR^7 forms a ring system selected from the group having the formula:
$-N(R^4)-CH_2-CH=CH-$ in the case of R^2 or R^5 together with NR^3, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more lower alkyl groups, preferably methyl groups; or
$-N(R^8)-CH_2-CH=CH-$ in the case of R^6 or R^9 together with NR^7, wherein at least one of the $-CH_2-$ or $-CH=$ groups of the ring system is optionally substituted by one or more lower alkyl groups, preferably methyl groups.

10. A compound as claimed in any one of the preceding claims wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are as defined in any one of the preceding claims provided that R^2 together with NR^3 and/or R^9 together with NR^7 form a ring system as defined in any one of the preceding claims.

11. A compound as claimed in any one of claims 1 to 9 wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are as defined in any one of claims 1 to 9 provided that R^5 together with NR^3 and/or R^6 together with NR^7 form a ring system as defined in any one of claims 1 to 9.

12. A compound as claimed in any one of claims 1 to 9 wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are as defined in any one of claims 1 to 9 provided that R^2 together with NR^3 and R^6 together with NR^7 form a ring system as defined in any one of claims 1 to 9.

13. A compound as claimed in any one of claims 1 to 9 wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are as defined in any one of claims 1 to 9 provided that R^5 together with NR^3 and R^9 together with NR^7 form a ring system as defined in any one of claims 1 to 9.

14. A compound as claimed in any one of claims 1 to 9 wherein:

R^1, R^6, R^9 and R^{10} are as defined in any one of claims 1 to 4;

R^4 represents H or lower alkyl;

R^2, R^7 and R^8 each represent lower alkyl; and

R^5 together with NR^3 forms a ring system as defined in any one of claims 1 to 9.

15. A compound as claimed in claim 14 wherein:

R^1, R^4, R^6, R^9 and R^{10} independently represent H or methyl; and

R^2, R^7 and R^8 each represent methyl.

16. A compound as claimed in claim 14 or 15 wherein R^4 represents H.

17. A compound as claimed in any one of claims 1 to 9 wherein:

R^1, R^2, R^5, R^6, R^9 and R^{10} are as defined in any one of claims 1 to 4;

R^4 and R^7 independently represent H or lower alkyl;

R^8 represents lower alkyl; and

provided that R^2 together with NR^3 or R^5 together with NR^3 form a ring system as defined in any one of claims 1 to 9.

18. A compound as claimed in claim 17 wherein R^8 represents methyl.

19. A compound as claimed in claim 17 or 18 wherein R^7 represents H or methyl.

20. A compound as claimed in any one of claims 17 to 19 wherein R^4 represents H or lower alkyl.

21. A compound as claimed in claim 20 wherein R^4 represents H.

22. A compound as claimed in any one of claims 17 to 21 wherein R^2 together with NR^3 forms a ring system selected from $-N(R^4)-(CH_2)_2-$ or $-N(R^4)-(CH_2)_3-$ and R^5 independently represents H or lower alkyl.

23. A compound as claimed in any one of claims 17 to 22 wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H or methyl.

24. A compound as claimed in claim 11 wherein R^2, R^4, R^8 and R^9 each represent lower alkyl; R^3 and R^7 independently represent H provided that R^5 together with NR^3 and/or R^6 together with NR^7 form a ring system as defined in any one of claims 1 to 9.

25. A compound as claimed in claim 24 wherein:
R^2 and R^9 each represent methyl; and
R^4 and R^8 each represent ethyl.

26. A compound as claimed in any one of claims 1 to 5 wherein:

R^3 represents H;

R^7 represents lower alkyl;

R^4 and R^8 independently represent H or lower alkyl; and

R^1, R^2, R^5, R^6, R^9 and R^{10} are as defined in any one of claims 1 to 5, provided that at least one of R^1, R^2, R^5, R^6, R^9 or R^{10} is other than hydrogen.

27. A compound as claimed in claim 26 wherein R^7 represents methyl.

28. A compound as claimed in claim 26 or 27 wherein R^8 represents methyl.

29. A compound as claimed in any one of claims 26 to 28 wherein R^4 represents H.

30. A compound as claimed in claim 29 wherein R^2 represents methyl.

31. A compound as claimed in claim 26 or 27 wherein R^8 represents H.

32. A compound as claimed in any one of claims 26 to 28 wherein R^4 represents methyl.

33. A compound as claimed in claim 31 wherein R^4 represents H.

34. A compound as claimed in any one of claims 26 to 29 and 31 to 33 wherein R^1, R^2, R^5, R^6, R^9 and R^{10} independently represent H or methyl.

35. A compound as claimed in claim 30 wherein R^1, R^5, R^6, R^9 and R^{10} independently represent H or methyl.

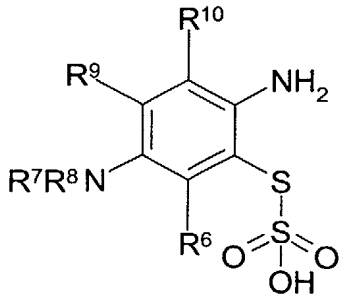
36. A compound as claimed in any one of claims 1 to 5 wherein:
R^3 and R^7 each represent H;
R^4 and R^8 each represent ethyl;
R^2 and R^9 each represent methyl; and
R^1, R^5, R^6 and R^{10} independently represent H or lower alkyl.

37. A compound as claimed in claim 36 wherein R^1, R^5, R^6 and R^{10} independently represent H or methyl.

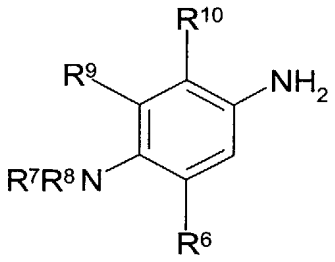
38. A compound as claimed in any one of claims 1 to 6 wherein at least one of R^3 together with R^4 and R^7 together with R^8 forms a ring system having the formula $-(CH_2)_n-X-(CH_2)_m-$ as defined in claim 1, and provided that at least one of R^3, R^4, R^7 or R^8 represents H.

39. A compound as claimed in any one of claims 1 to 6 and claim 38 wherein R^7 together with R^8 forms a ring system as defined in claim 38 and R^3 and R^4 both represent H.

40. A method of preparing a compound of formula I as defined in any one of claims 1 to 39 which comprises reacting a compound of formula II or of formula IV

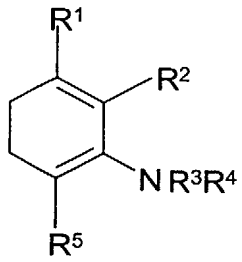


II



IV

with a compound of formula III



III

wherein R^1, R^2, R^3, R^4, R^5, R^6, R^7, R^8, R^9 and R^{10} are as defined in any one of the preceding claims.

41. A composition comprising an insoluble support and a compound of formula I as defined in any one of claims 1 to 39.

42. A composition as claimed in claim 41 wherein the compound of formula I is fixed to the surface of the insoluble support.

43. A composition as claimed in claim 41 or 42 wherein the compound of formula I is covalently bonded to the insoluble support.

44. A composition as claimed in any one of claims 41 to 43 wherein the insoluble support is a polymer.

45. A composition as claimed in claim 44 wherein the polymer forms a flexible or a rigid support.

46. A method of making a composition as claimed in any one of claims 41 to 45 which comprises forming an insoluble support including a compound of formula I as defined in claims 1 to 39.

47. A compound of formula I as defined in claims 1 to 39 or a composition as claimed in claims 41 to 45 for use in medicine.

48. Use of a compound of formula I as defined in any one of claims 1 to 39 in the manufacture of a medicament for the prophylactic and/or curative treatment of a pathogenic infection.

49. The use as claimed in claim 48 wherein the pathogenic infection is a bacterial infection.

50. Use of a compound of formula I as defined in any one of claims 1 to 39 or a composition as claimed in claims 41 to 45 for reducing the level of a pathogenic contaminant, such as a virus, a bacterium and a parasite.

51. The use as claimed in claim 50 wherein the pathogenic contaminant comprises Gram-negative and/or Gram-positive bacteria.

52. A method for reducing the level of a pathogenic contaminant which comprises delivering a compound of formula I as defined in any one of claims 1 to 39 or a composition as claimed in claims 41 to 45 to the pathogenic contaminant; and irradiating the compound or composition with light sufficient to generate singlet oxygen.

53. A method as claimed in claim 52 wherein the pathogenic contaminant is present in whole blood and/or a blood component of whole blood.

54. A method as claimed in claim 52 or 53 wherein the pathogenic contaminant comprises Gram-negative and/or Gram-positive bacteria.

55. A composition comprising whole blood and/or a component of whole blood and a compound of formula I as defined in any one of claims 1 to 39 or a composition as claimed in any one of claims 41 to 45.

56. A pharmaceutical composition comprising a compound of formula I as defined in any one of claims 1 to 39 in admixture with a pharmaceutically acceptable adjuvant, diluent or carrier.

57. The pharmaceutical composition as claimed in claim 56 for topical application to a target tissue.

58. Use of a compound as defined in any one of claims 1 to 39 in the manufacture of a medicament for reducing the level of pathogenic contaminants in whole blood and/or a component of whole blood.

59. The use as claimed in claim 58 wherein the pathogenic contaminant comprises Gram-negative and/or Gram-positive bacterium.

60. The use as claimed in claim 58 or 59 wherein the component of whole blood comprises red blood cells.

61. Use of a compound as claimed in any one of claims 1 to 39 in the manufacture of a medicament for use as a topical antimicrobial agent.

62. A medical device including a compound as claimed in any one of claims 1 to 39 or composition as claimed in any one of claims 41 to 45.

63. Use of a compound of formula I as claimed in any one of claims 1 to 39 or a composition as claimed in any one of claims 41 to 45 or 56 for producing light induced reactive oxygen species, such as singlet oxygen.

64. A method of producing light-induced reactive oxygen species, such as singlet oxygen, which comprises irradiating a compound as claimed in any one of claims 1 to 39 or a composition as claimed in any one of claims 41 to 45 or 56 with light in the presence of oxygen.

65. A dyestuff or surface coating material comprising a compound as defined in any one of claims 1 to 39.

66. Use of the dyestuff or surface coating material as defined in claim 65 for forming a hygienic coating.

INVESTOR IN PEOPLE

Application No: GB 0105730.6 **Examiner:** Dr Annabel Ovens
Claims searched: 1-66 **Date of search:** 25 July 2002

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Search Report under Section 17

Databases searched:

UK Patent Office collections, including GB, EP, WO & US patent specifications, in:

UK Cl (Ed.T): C2C

Int Cl (Ed.7): C07D 279/18, 513/02

Other: Online: PAJ, EPODOC, WPI, CAS-ONLINE

Documents considered to be relevant:

Category	Identity of document and relevant passage		Relevant to claims
X, P	WO 01/96322 A1	(GALEY) see page 4 line 16-page 5 line 21, page 6 lines 6-12, page 10 line 4-page 13 line 33 and Examples	1 at least
X	WO 98/31219 A1	(AMERICAN NATIONAL RED CROSS) see page 4 line 16-page 5 line 8 and page 13 line 9-page 14 line 4	1 at least
A	WO 93/21992 A1	(INSTITUTE OF DENTAL SURGERY)	
A	US 5830526	(WILSON AND BULL)	

X Document indicating lack of novelty or inventive step
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